DETERMINATION OF HUMAN PAPILLOMA VIRUSES DNA AND GENOTYPES IN GENITAL SAMPLES WITH PCR

GENİTAL ÖRNEKLERDE HUMAN PAPİLLOMA VİRUS DNA VARLIĞININ VE GENOTİPLERİNİN PCR İLE ARAŞTIRILMASI

HPV TYPES IN GENITAL SAMPLES

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Servikal süründü ve biyopsi örneklerinde saptanan HPV tipleri.
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Öz
Amaç: Human Papilloma Virus'ün (HPV) dünya çapında her yıl 528.000 yeni vakaya ve 266.000 servikal kanser nedenli ölüme neden olduğu bildirilmektedir. HPV 16, enfeksiyonların %54.4'ünden, HPV 18 ise %16.5'inden sorumludur. Bu çalışmada Üniversitesi Tıp Fakültesi Hastanesi'ne başvuran kadınlarda genital HPV enfeksiyonunun prevelansı ve genotiplerin dağılımı araştırılmıştır. Gereç ve Yöntem: Bu çalışmada 261 genital örnekte HPV DNA, yüksek riskli 12 HPV genotipini (HPV16/18/31/33/35/39/45/51/52/56/58/59) saptayabilen ticari multipleks real-time PCR (Sacace Biotechnologies, İtalya) testi ile araştırılmıştır. Bulgular: 261 genital örneğin 100'ünde (%38.3) HPV DNA pozitifliği saptanmış olup kitin ile saptanabilen tüm genotiplere rastlanmıştır. Altmış bir örnekte (%61.0) tek HPV tipi, 39 örnekte (%39.0) ise birden fazla HPV tipi saptanmıştır. Çoklu genotip enfeksiyonlarında en sık iki tip (%53.8) ile enfeksiyon görülmüştür. HPV DNA pozitif örneklerde, en sık tip 16'ya (39/100,%39.0) rastlanırken, onu sırasıyla tip 51 (22/100,%22.0), tip 56 (18/100,%18.0), tip 52 (15/100,%15) ve tip 31 (15/100,%15) izlemiştir. Tek HPV tipi saptanan örneklerde en sık tip 16 (16/61,%26.2) daha sonra sırasıyla tip 51 (9/61,%14.8) ve tip 56 (8/61,%13.1) saptanırken, birden fazla HPV tipi saptanan örneklerde de tip 16 (23/39,%59.0) ve tip 51 (13/39,%33.3) ilk iki sırada yer alırken, onları tip 31 (11/39,%28.2) izlemiştir. Tartışma: Sonuç olarak, çalışma grubunu oluşturan kadınlarda genital HPV enfeksiyonu prevelansı %38.3 olarak belirlenmiş olup, dünyada olduğu gibi en sık saptanan HPV tip 16 olmuştur. HPV tip 18 diğer yüksek riskli HPV tipleri olan HPV tip 51 ve 56'dan daha geride yer almıştır. Bu tür epidemiyolojik çalışmalar servikal kanser tarama algoritmalarının geliştirilmesinde ve aşı çalışmalarında yol gösterici olmaktadır.

Anahtar Kelimeler
HPV; Servikal Sürüntü; Real-Time PCR; HPV Tiplendirme

Abstract
Aim: It is reported that each year HPV causes 528,000 new cases and 266,000 cervical cancer induced deaths worldwide. HPV16 is responsible for 54.4%, HPV18 is responsible for 16.5% of the infections. In this study, the prevalence of genital HPV infection and distribution of genotypes were investigated in women who admitted to University Hospital. Material and Method: Genital samples of 261 women were investigated for the high-risk 12 HPV genotypes (HPV16/18/31/33/35/39/45/51/52/56/58/59) with multiplex realtime PCR (Sacace Biotechnologies,Italy). Results: In 100 of 261 (38.3%) genital samples, HPV DNA were positive and all genotypes that can be detected by the kit were identified at least once. In 61 samples (61.0%) a single HPV type was identified and in 39 samples (39.0%) more than one genotype was identified. Most mixed infections (53.8%), occurred with two HPV types. In HPV DNA positive samples, the most common type was HPV16 (39/100,39.0%) followed by HPV51 (22/100,22.0%), HPV56 (18/100,18.0%), HPV52 (15/100,15.0%) and HPV31 (15/100,15.0%). The most common genotypes identified in samples infected with a single type were HPV16 (16/61,26.2%), HPV51 (9/61,14.8%) and HPV56 (8/61,13.1%). In mixed infections, HPV16 (23/39,59.0%) was followed by HPV51 (13/39,33.3%) and HPV31 (11/39,28.2%). Discussion: As a conclusion, the prevalence of genital HPV infection in the studied population of women was 38.3%. HPV16 was the most common type similar to the worldwide data, while HPV18 was less prevalent than the other high-risk HPV types, HPV51 and HPV56. Such epidemiologic studies are useful to guide development of cervical cancer screening algorithms and vaccination studies.

Keywords
HPV; Cervical Swab; Real-Time PCR; HPV Typing

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Introduction

Human papillomaviruses (HPV), which have been proven to cause cervical cancer, are enveloped, double-stranded, icosahedral, symmetrical DNA viruses classified in Papillomaviridae. HPV induced cervical cancer is the fourth most prevalent cancer type in women worldwide and accounts for 12% of all cancers in women. It is reported that HPV causes 528,000 new cases worldwide and 266,000 cervical cancer deaths each year [1].

In less developed countries such as in Africa, cervical cancer is the most common type of cancer in women while the prevalence of HPV decreases in more developed communities, such as European countries [2,3]. Sub-Saharan Africa is the most prevalent region for cervical cancer with at prevalence of up to 33.6%. The prevalence of HPV-associated cervical cancer in Asian countries has been reported as 9.4% [3]. In the GLOBOCAN study published by the International Cancer Agency (IARC), the incidence of cervical cancer in Turkey is 4.31 per 100,000 [1]. According to data from the Turkish Public Health Agency Cancer Center, cervical cancer in Turkey is the tenth most common cancer among female cancers and constitutes 4.6% of all cancers in women.

It is difficult to determine the true prevalence and distribution of HPV types in our country since studies are performed in different small study groups and using different techniques. There are three main methods for the diagnosis of HPV: thus some differences in results could be due to the method used in the studies. The methods are direct hybridisation methods (southernblot, dotblot, in situ hibridisation), signal amplification test (Hibridcapture II) and the nucleic acid amplification test (PCR). The sensitivity, specificity and NPV of PCR-based HPV DNA detection methods are higher than for the other techniques [4].

There are more than 40 HPV genotypes that cause genital infections [5]. In terms of causing cancer potentials, HPV types are classified as low-risk types (HPV 6, 11, 40, 42, 43, 44, 53, 54, 61, 72, 81) and high-risk types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82) [6,7]. Worldwide, HPV 16 is responsible for 54.4% of infections and HPV 18 is responsible for 16.5%. In 70% of all invasive cervical cancers the causative agent is HPV 16 and 18 [8,9].

Currently, there are three approved HPV vaccines available for the prevention of cervical cancer caused by HPV. Existing vaccines are not protective against all HPV types. These vaccines are two-valent HPV 16/18 (Cervarix® GlaxoSmithKline, UK), quadrivalent HPV 16/18/6/11 (Gardasil® Merck & Co, USA) vaccines, and a second generation vaccine Gardasil 9® (HPV 6,11,16,18,31,33,45,52,58) containing nine HPV types [10,11]. Determination of the distribution of HPV types in a specific community is an important consideration in determining vaccination policies.

In this study, the distribution of HPV types and the prevalence of genital HPV infection in women who were admitted to Dokuz Eylül University Hospital Obstetrics and Gynecology Clinic were investigated and results were compared with other study results.

Material and Method

In this study, the prevalence of genital HPV infection and distribution of HPV types in women who were admitted to Obstetrics and Gynecology Clinic of Dokuz Eylül University Hospital were investigated. The study group consisted of 261 female patients, all within the age range of 19-65 (mean age 35.1 ± 9.7), who were admitted to the Obstetrics and Gynecology Clinic between 2009 and 2011.

261 cervical smears and biopsies, were investigated for the 12 high-risk HPV genotypes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) with multiplex real-time PCR test (Sacace Biotechnologies, Italy) a real-time amplification test for qualitative detection and typing of stated HPVgenotypes. The test is based on two major processes: isolation of DNA from specimes and multiplex real-time amplification of four tubes for each sample. Each tube contains primers directed against regions of three HPV types and the ß-globine gene used as internal control.

Results

HPV DNA was positive (mean age:33.7±9.1) in 100 of 261 (38.3%) cervical samples. In 61 samples (61.0%) a single HPV type was identified, in 39 samples (39.0%) more than one HPV type (mixed infections) was identified. Most mixed infections, (21/39, 53.8%) occurred with two HPV types. In 100 samples, a total of 172 HPV types were identified; 61 of these were from the samples containing one HPV type, and 111 were obtained from samples containing multiple HPV types (Table 1).

Type distribution in HPV types with single and multiple HPV-DNA with mean age are shown in Table 2.

In HPV DNA positive samples, the most common type identified was HPV 16 (39/100, 39.0%) followed by HPV type 51 (22/100, 22.0%) and HPV type 56 (18/100, 18.0%). When samples with singleplex or multiplex infection were examined separately, it was found that in the samples infected with one type, the most

Table 1. Results of HPV DNA and type distribution in 261 cervical samples

HPV Types	HPV DNA positive samples n(%)	Infection with single HPV type n(%)	Infection with multiple HPV types n(%)	HPV DNA negative samples n(%)
Type16	39 (39/100, 39.0%)	16 (16/61, 26.2%)	23 (23/39, 59.0%)	-
Type 51	22 (22/100, 22.0%)	9 (9/61, 14.8%)	13 (13/39, 33.3%)	-
Type 56	18 (18/100, 18.0%)	8 (8/61, 13.1%)	10 (10/39, 25.6%)	-
Type 52	15 (15/100, 15.0%)	5 (5/61, 8.2%)	10 (10/39, 25.6%)	-
Type 31	15 (15/100, 15.0%)	4 (4/61, 6.6%)	11 (11/39, 28.2%)	-
Type 39	13 (13/100, 13.0%)	7 (7/61, 11.4%)	6 (6/39, 15.4%)	-
Type 18	13 (13/100, 13.0%)	5 (5/61, 8.2%)	8 (8/39, 20.5%)	-
Type 59	11 (11/100, 11.0%)	1 (1/61, 1.6%)	10 (10/39, 25.6%)	-
Type 35	9 (9/100, 9.0%)	2 (2/61, 3.3%)	7 (7/39, 17.9%)	-
Type 45	7 (7/100, 7.0%)	2 (2/61, 3.3%)	5 (5/39, 12.8%)	-
Type 33	5 (5/100, 5.0%)	1 (1/61, 1.6%)	4 (4/39, 10.3%)	-
Type 58	5 (5/100, 5.0%)	1 (1/61, 1.6%)	4 (4/39, 10.3%)	-
Total number of samples (n:261)	100 (100/261, 38.3%)	61 (61/261, 23.4%)	39 (39/261, 14.9%)	161 (161/261, 61.7%)
Total number of genotypes (n:172)	172 (172/172, 100.0%)	61(61/172, 35.5%)	111 (111/261, 64.5%)	-

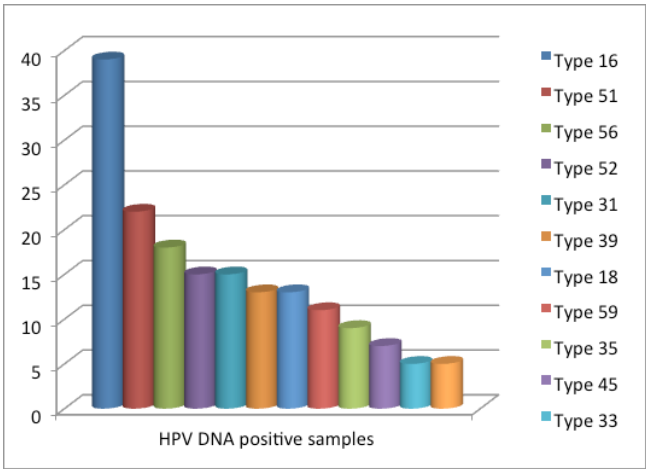

Figure 1. Distribution of HPV types in HPV DNA positive samples

common types were HPV 16 (16/61, 26.2%), HPV 51 (9/61, 14.8%) and HPV 56 (8/61, 13.1%). In mixed infections HPV 16 (23/39, 59.0%) was most common, followed by HPV 51 (13/39, 33.3%) and HPV 31 (11/39, 28.2%) (Table 1 and Figure 1).

Discussion

After persistent infection with high-risk HPV types, it takes a long time to develop cervical cancer. Prevention or early detection of cervical cancer is possible by the application of various screening programs (Pap smear and / or HPV DNA detection) and preventive health services (HPV vaccination). In many European countries, such as the United Kingdom, Finland, Italy and the Netherlands, it is reported that screening, follow-up and protection programs reduce the incidence and mortality of HPV [12].

The role of HPV types in the transformation of cervical cancer is well known. For this reason, besides the presence of HPV DNA, genotyping has a critical role. It is important to identify the distribution of HPV types and infections caused by multiple types to improve disease management and vaccination programs. The prevalence of HPV, the distribution of HPV types, and the frequency of multiple infections vary according to the region and the characteristics of the study groups [13].

Since studies on HPV infections in Turkey are conducted in limited populations and centers, it is difficult to determine the actual prevalence and distribution of HPV types. The National HPV screening project, targeting women aged 30-65, has recently been initiated by the Ministry of Health, Public Health Agency, Cancer Department. According to the first published results, 839,756 women were screened and HPV DNA positivity was detected in 29,240 (3.48%) of them.

In this study, we aimed to contribute to our country's data by investigating the prevalence of genital HPV infection and the distribution of HPV types in our study group.

HPV DNA was detected in 38.3% (100/261) of the samples examined in our study. 61 (61.0%) of HPV DNA positive specimens had a single HPV type and 39 (39.0%) had more than one HPV type (Table 1 and 2). When compared with studies of similar patient groups and methods, HPV positivity was higher in our study.

HPV DNA positivity has been reported to range from 3.2% to 30.6% in studies investigating the presence of HPV by the PCR method in patients admitted to different outpatient clinics in Turkey (Table 3) [14-19]; this rate increases to a range of 57.5% to 70.0% in patients with cytologic atypia [20-22].

In our study, the most frequent type was HPV 16 (39.0%) followed by type 51 (22.0%), type 56 (18.0%), type 52 (15.0%) and type 31 (15.0%) (Table 1). HPV type 16 was also found to be the most common type in different epidemiological studies in Turkey, similar to our findings. However, the types following HPV 16 differ between studies (Table 3) [14-20]. In the study conducted by Unal et al. [18] HPV type 16, type 56 and type 51 were the most common three types respectively, similar to the

Table 2. Co-infection patterns and genotype distribution

Infection with single HPV type (n:61) (mean age)	Two HPV types (n:21) (mean age)	Three HPV types (n:10) (mean age)	Four HPV types (n:3) (mean age)	Five HPV types (n:4) (mean age)	Seven HPV types
Type 16 (n:16) 37.2±13.3)	Type 16,18 (n:2) (31.0±5.7)	Type 39,45, 51 (n:1)(25.0)	Type 31,39,51,52 (n:1)(37.0)	Type 16,31,45, 52,59 (n:1) (31.0)	Type16,18,31,35,45,51,58 (n:1) (24.0)
Type 51 (n:9) (33.1±8.8)	Type 16,33 (n:2) (40.0±14.1)	Type 16,31, 51 (n:1)(27.0)	Type 31,33,52,59 (n:1)(22.0)	Type 18,45,51, 52,59 (n:1) (38.0)	
Type 56 (n:8) (33.1±6.3)	Type 16,35 (n:2) (37.5±5.0)	Type 51,56, 58 (n:1)(24.0)	Type 16,18,31,56 (n:1)(39.0)	Type 16,33,52, 56,59 (n:1) (27.0)	
Type 39 (n:7) (38.7±6.8)	Type 16,51 (n:2) (34.5±0.7)	Type 18,56, 59 (n:1)(35.0)		Type 16,31,39, 51,56 (n:1) (35.0)	
Type 52 (n:5) (27.4±5.7)	Type 16,31 (n:1) (22.0)	Type 16,35, 52 (n:1)(34.0)			
Type 18 (n:5) (42.4±12.8)	Type 16,39 (n:1) (27.0)	Type 18,45, 51 (n:1)(24.0)			
Type 31 (n:4) (32.3±9.4)	Type 16,56 (n:1) (27.0)	Type 16,51, 52 (n:1)(34.0)			
Type 35 (n:2) (24.5±0.7)	Type 16,59 (n:1)(39.0)	Type 16,58, 59 (n:1)(23.0)			
Type 45 (n:2) (27.5±9.2)	Type 18,56 (n:1) (35.0)	Type 16,35, 58 (n:1)(39.0)			
Type 59 (n:1) (41.0)	Type 39,56 (n:1) (35.0)	Type 16,39, 59 (n:1) (37.0)			
Type 33 (n:1) (31.0)	Type 51,59 (n:1) (33.0)				
Type 58 (n:1) (28.0)	Type 52,59 (n:1) (39.0)				
	Type 31,51 (n:1) (24.0)				
	Type 35,52 (n:1) (29.0)				
	Type 31,56 (n:1) (47.0)				
	Type 35,56 (n:1) (28.0)				

Table 3. Prevalence of HPV DNA and genotype distribution identified by PCR based assays in outpatient studies from Turkey

Study	Region/year	Study group (n)	Age (mean age)	HPV-DNA Positivity	Infection with multiple HPV types	The most frequent HPV types
Polat et al [14]	Ankara/2009	403	19-67 (37.5)	93 (23.0%)	(11.1%)	Tip 16 (36.0%)
						Tip 6 (22.0%)
						Tip 18 (13.0%)
						Tip 11 (4.4%)
						Tip 45 (4.4%)
Altun et al [15]	Adana/2011	460	20-68	24 (5.2%)	5 (5/24, 20.8%)	Tip 16 (33.3%)
						Tip 45 (20.8%)
						Tip 18 (4.2%)
						Tip 31 (4.2%)
Şahiner et al [16]	Ankara/2012	356	16-64 (38.0)	109 (30.6%)	29 (29/101, 28.7%)	Tip 16 (33.7%)
						Tip 52 (12.6%)
						Tip 58 (11.6%)
						Tip 18 (7.4%)
						Tip 31 (7.4%)
						Tip 35 (7.4%)
Yüce et al [17]	Ankara/2012	890	20-70 (39.5)	229 (25.7%)	54 (54/229, 23.6%)	Tip 16 (46.3%)
						Tip 31 (17.0%)
						Tip 51 (17.0%)
						Tip 42 (8.3%)
						Tip 33 (7.9%)
Ünal et al [18]	Antalya/2013	1137	20-66	36 (3.2%)		Tip 16 (22.0%)
						Tip 56 (13.5%)
						Tip 51 (11.8%)
						Tip 31 (8.5%)
						Tip 59 (8.5%)
Polat et al [19]	Multicenter study/2013	6388	15-76 (38.9)	1588 (25.0%)	8 (8/1588, 0.5%)	Tip 16 (32.0%)
						Tip 6 (17.0%)
						Tip 11 (9.0%)
						Tip 18 (8.0%)
						Tip 31 (6.6%)

results in our study. As in some other studies, HPV type 18 was less common than the other high-risk HPV types in our findings [17,18,20].

In a meta-analysis consisting of 1,016,719 women and 194 trials using PCR or Hybrid Capture 2 methods, HPV prevalence was reported as 11.7% globally and 1.7% in Turkey. The most common genotypes worldwide were HPV 16 (3.2%), HPV 18 (1.4%), HPV 52 (0.9%), HPV 31 (0.8%), HPV 58 (0.7%) and HPV 39 (0.6%), HPV 56 (0.6%), HPV 53 (0.6%), and HPV 51 (0.6%) at equal prevalence. However, it has been emphasized that the distribution of genotypes varies between regions. In the Asian region in which Turkey is located, HPV 16 (2.5%), HPV 18 (1.4%), HPV 52 (0.7%) infections were reported and HPV 51 (0.5%), HPV 58 (0.5%), HPV 53 (0.5%), HPV 56 (0.5%) were at equal prevalance; HPV 39 infections were reported at 0.4% [3]. Although the distribution of HPV genotypes varies between regions, HPV 16 infection is most common in all regions [3,9].

Existing vaccines are not protective against all HPV types. The last FDA approved vaccine is nine-valent Gardasil 9® which currently has the widest coverage, containing nine HPV types (HPV 6, 11, 16, 18, 31, 33, 45, 52, 58). It will be appropriate to revise the effectiveness of the current vaccines over the years and to develop vaccine applications according to the needs of the HPV genotype distribution.

The prevalence of infection with multiple HPV genotypes also varies according to the regions. The frequency of mixed infection was reported in the range of 0.5-28.7% in an outpatient population screened by PCR-based assays in Turkey (Table 3). In patients with cytologic atypia this rate is higher, at 45.5%. Worldwide studies report mixed infections as nearly 40% of all HPV infections [23]. In a study conducted by Clifford et al. [24], the frequency of mixed infections was reported as 11.5-42.4% using the same method as used in different regions. In our study, the frequency of mixed infections was determined to be 39.0% with at most two different HPV types (53.8%). Multiple infections with three, four, five and seven HPV types were detected at decreasing rates (Table 2). The prevalance of mixed infection in our study is higher than the similar studies (0,5%-28,7%) reported from Turkey (Table 3).

It is stressed that multiple HPV infections may be higher in HIV-infected individuals and those with advanced cytologic atypia [25]. It is also emphasized that mixed HPV infection may be associated with persistence although. However, the interaction of different HPV types detected in mixed infections and the effect of this interaction on cervical cancer transformation has not yet been fully elucidated [13].

As a summary, HPV DNA was positive in 38.3% of our study population with 39% being mixed infection. The most common type was HPV 16 which is similar to data gathered from other Turkish studies. There are differences between the frequency and order of the HPV genotypes detected at the second and subsequent frequencies. It is difficult to determine the true prevalence and distribution of HPV types in Turkey since studies are generally conducted in small groups and at certain centers. The results of the community-based "Cervical Cancer Screening Program" conducted by the Public Health Agency Cancer Center will be provide guidance for further investigation.

Competing interests

The authors declare that they have no competing interests.

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